Relative and Absolute Photorefractoriness in Turkey Hens: Profiles of Prolactin, Thyroxine, and Triiodothyronine Early in the Reproductive Cycle

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ABSTRACT An experiment was conducted to determine whether a commercial strain of turkey hens exhibits relative photorefractoriness (rPR) during a reproductive cycle and to ascertain whether plasma levels of certain hormones early in the reproductive cycle might be associated with subsequent expression of rPR or absolute photorefractoriness (aPR). Twenty-seven percent of hens maintained on a stimulatory photoperiod of 18L:6D for 19 wk and then given a shorter, but still stimulatory, photoperiod (13L:11D) ceased to lay and their ovaries regressed within 4 wk. These hens were considered rPR. Subsequent exposure to the 18L:6D photoperiod resulted in ovarian recrudescence in 41.7% of these PR individuals, confirming the presence of rPR at 19 wk after photostimulation. Absolute PR was observed in 15.1% of hens during a 27-wk reproductive season. Hens that became rPR or aPR exhibited significantly lower plasma prolactin levels at 8 and 14 wk after photostimulation than did hens that remained photosensitive (PS). Plasma levels of thyroxine were lower at 1 and 2 wk following photostimulation in hens that subsequently became PR than in hens that remained PS.

We conclude that turkey hens may exhibit rPR and aPR during a reproductive cycle, whereas flockmates may remain PS for at least 27 wk. The presence of long daylengths, thyroid hormones, and PRL did not assure expression of PR. The expression of PR appears to be associated with reduced plasma throxine levels during a period when programming of PR is thought to occur and with reduced levels of prolactin following peak egg production.

(*Key words*: Turkey, photorefractoriness, prolactin, thyroid hormone)

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INTRODUCTION

The turkey is a seasonal breeding bird. Reproduction is controlled by photoperiod and is a balance between two physiological states, photosensitivity (PS) and photorefractoriness (PR). The current concept of PR, reviewed by Sharp (1993; 1996) for chickens and other birds, is that photostimulation with a photoperiod that is longer than the critical day length initiates not only the neural inputs that stimulate gonadotropin secretion but additionally initiates the progressive development of inhibitory inputs to chicken gonadotropin-releasing hormone-I (GnRH-I) neurons that eventually shut down reproduction. When the inhibitory inputs exceed the stimulatory inputs to GnRH-I neurons, the system shuts down and reproduction ceases. If an increase in photoperiod cannot overcome this inhibition, then the bird is said to be absolutely PR (aPR). However, it is possible that these inhibitory inputs may be so weak as to have no effect on overt reproduction in the presence of the stimulation of a conventional long photoperiod (generally between 14L:12D and 18L:6D).

The presence of these weak and covert inhibitory inputs can then only be observed by reducing the photoperiodic drive until it is close to or below the critical daylength. The inhibitory inputs can then predominate and terminate reproduction under a photoperiod that would normally be stimulatory (Sharp, 1993). Because these inhibitory inputs are weak, a subsequent increase in photoperiod may then overcome the inhibition and the bird will return to reproduction. This latent inhibition of GnRH-I is termed relative PR (rPR). It has been suggested that rPR is a lesser form of, and precedes, aPR (Follett and Nicholls,

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Abbreviation Key: aPR = absolute photorefractoriness; GnRH-I = chicken gonadotropin-releasing hormone-I; LH = luteinizing hormone; PR = photorefractory, photorefractoriness; PRL = prolactin; PS = photosensitive, photosensitivity; rPR = relative photorefractoriness; T3 = triiodothyronine; T4 = thyroxine.

1984; Nicholls et al., 1988; Bentley et al., 1997). Turkey hens, unlike chickens, have generally been thought to express aPR only, but recently studies (Siopes, 2001) have suggested that turkey hens may also express rPR. An objective of the current research was to definitively test for rPR in laying turkey hens.

The identity is not known for the inhibitory inputs that terminate reproduction. However, the onset of aPR is thought to be programmed at the time of photostimulation or shortly thereafter. Extensive studies using sparrows (Wilson and Reinert, 2000; Wilson, 2001) and starlings (Bentley et al., 2000; Dawson et al., 2001) suggest that the onset of PR is programmed by the presence of thyroid hormone and long daylengths within a few weeks after photostimulation. Thyroxine (T4) is thought to be the thyroid hormone responsible for this programming (Wilson, 2001). Earlier studies also linked increases in circulating prolactin (PRL) levels with onset of PR (Dawson et al., 1983), but this link has been discounted in more recent studies (reviewed by Dawson et al., 2001). Only one study has been conducted to compare circulating levels of triiodothyronine (T3), T4, and PRL following photostimulation in relation to whether a bird remains PS or becomes PR later in the reproductive season. This study (Lien and Siopes, 1989) found that turkey hens that subsequently became PR had markedly decreased PRL levels beginning 11 wk after photostimulation than hens that did not become PR. No differences in T3 or T4 were observed between PR and PS hens, but no sampling was done between 0 and 2 wk of photostimulation, which is now thought to be a critical time for programming of PR. Consequently, a second objective of our research was to compare, retrospectively, the early post-lighting hormone profiles of these hormones in hens that remained PS vs. those that became rPR or aPR.

MATERIALS AND METHODS

Birds

Female parent line BUTA[2] Strain 37 roaster turkeys were raised from 1 d of age following the guidelines of the primary breeder. Birds were raised on a 14L:10D photoperiod until 18 wk of age and then on a 6L:18D photoperiod until 30 wk of age. Hens were moved at 29 wk of age to laying pens in two rooms with independent light control of sodium vapor lamps set to deliver 50 lx at bird height. They were photostimulated at 30 wk of age with a photoperiod of 18L:6D (lights on 0500 h). This was a spring flock, with hens photostimulated in mid-April. Egg production and nesting activity were monitored by trap-nesting, with the nests checked and hens expelled five times per day. Any hens that became broody or that did not lay consistently in the trapnests were removed from the experiment. Five-milliliter blood samples were collected into heparinized tubes from all hens via the ulnar vein in the morning (0700 to 1100 h) at 0, 1, 2, 8, and 14 wk following photostimulation. Plasma was separated and stored at –70 C for hormone analysis. At 19 wk of lay, one room of hens (n = 69) was changed to a photoperiod of 13L:11D (on at 0500 h), while the other (n = 48) remained on 18L:6D. After 4 wk, hens that had been laying under the 18L:6D photoperiod and had ceased laying (but were not incubating) under 13L:11D were deemed to be rPR (n = 17). These rPR hens had not laid for at least the final 11 d of the 13L:11D treatment. Hens that had remained on 18L:6D and had spontaneously ceased laying were deemed aPR (n = 11). At this time (23 wk after photostimulation), five hens that had ceased laying on the reduced photoperiod (rPR) and five that had continued laying under the short photoperiod (PS) were euthanized, and their ovaries examined and weighed. Five aPR hens (on 18L:6D) were also euthanized before collection of ovaries. Twelve of the remaining rPR hens were moved to the control room (18L:6D) for an additional 4 wk. Relatively PR hens that failed to resume laying after return to the 18L:6D photoperiod were deemed to have made a transition from rPR to aPR, whereas those that resumed lay were confirmed rPR by 27 wk of production. A flow chart of lighting changes and bird numbers is presented in Figure 1. At the end of the experiment, the ovaries of all PR hens, as well as five hens that continued laying under 13L:11D (definitively non-refractory) were inspected and weighed at necropsy to further assess physiological state. Hormone analyses were conducted only on blood samples from the hens in these rPR (n = 5), aPR (n = 11) and PS (n = 10) groups.

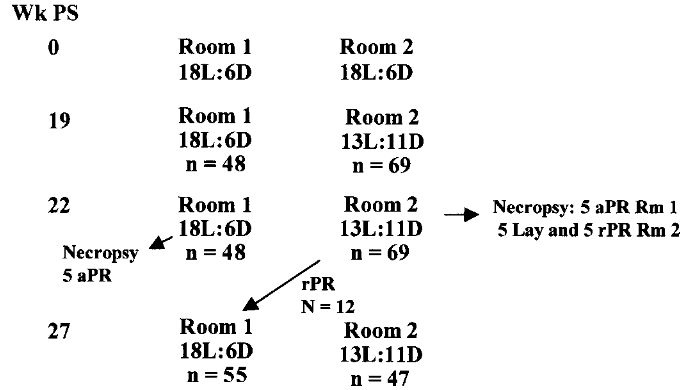

FIGURE 1. Flow chart of lighting changes and disposition of all experimental hens from photostimulation at 30 wk of age through 27 wk of production. Between 0 and 19 wk after photostimulation, broody hens and hens that did not lay in nests were removed. At 23 wk, five laying hens and five relatively photorefractory (rPR) hens from Room 2, and five absolutely photorefractory (aPR) hens from Room 1, were killed. Twelve additional rPR hens were moved from Room 2 to Room 1 at 23 wk. PS = photosensitive.

Hormone Assays

Plasma levels of luteinizing hormone (LH), PRL, T4 and T3 were measured by RIA. Prolactin was measured by using the homologous RIA of Proudman and Opel

[2]British United Turkeys of America, Lewisburg, WV.

TABLE 1. Ovary weights of representative hens after 23 wk of photostimulation[1]

Group	n	Ovary weight (g; $\bar{x} \pm$ SEM)
Photosensitive	5	121.45 ± 5.96^a
Relatively photorefractory	4	9.31 ± 0.50^b
Absolutely photorefractory	5	6.86 ± 0.85^b

[1]Photosensitive hens and relatively photorefractory hens received 18L:6D for 19 wk and then 13L:11D for 3 wk. Absolutely photorefractory hens received 18L:6D for 23 wk. Means followed by different letters are significantly different ($P \leq 0.05$).

(1981). The intra-assay and inter-assay CV were 1.6 and 6.4%, respectively. LH was measured by using a chicken LH RIA as described by Bacon and Long (1996). The intra-assay and inter-assay CV were 14.% and 19.0%, respectively. T4 and T3 were measured using commercial kits[3] with modifications for avian plasma described by Siopes (1997). The intra-assay and inter-assay CV of the T3 RIA were 17.8 and 11.9%, respectively, whereas those of the T4 RIA were 9.5 and 3.5%, respectively.

Statistical Analyses

Both one-way and repeated measures analyses of variance were used to evaluate treatment effects using the general linear models procedures of the SAS Institute (1990). Repeated measures analysis of variance was applied to the hormone data through 2 wk of photostimulation. The least squares mean option was used to estimate significant differences among treatment means. Statements of statistical significance were based on $P < 0.05$ unless specified otherwise.

RESULTS

Absolute PR under a 18L:6D photoperiod was observed as early as 12 wk following photostimulation. Eleven of 117 hens (9.4%) became aPR within 19 wk after photostimulation. Seven additional hens became aPR after exposure to 13L:11D for a total of 15.4% aPR hens for the 27-wk reproductive period. Among the 69 laying hens that were exposed to a reduced photoperiod (13L:11D) from 19 to 23 wk, 19 (27.5%) became PR. The ovaries of four rPR hens euthanized at this time were regressed, comparable in weight to those of five hens that were aPR on the 18L:6D photoperiod (Table 1). The fifth putative rPR hen was removed from the experiment at necropsy because of an abnormal reproductive tract. Of those rPR hens that were returned to 18L (n = 12), five (41.7%) resumed laying and seven became aPR. The hen-day egg production of PS, rPR, and aPR hens for the 27-wk production cycle is shown in Figure 2. The PS hens shown in this Figure are only those that remained PS on the 13L:11D photoperiod from 19 to 27 wk and were randomly selected for hormone analysis. By laying eggs on 13L:11D, these hens were demonstrably free of aPR and rPR and thus were ideal PS hens. However, the egg production curve of hens that remained PS on 18L:6D was similar (data not shown). The egg production of hens that became rPR or aPR did not differ significantly from the PS hens during the first 12 wk following photostimulation (data not shown).

Figure 3 compares the plasma concentrations of T3, T4, and the T3/T4 ratio of hens that remained PS on 13L:11D with those that became aPR on an 18L:6D photoperiod. Also shown are the hormone levels of hens that were definitively rPR (ceased laying on 13L:11D and resumed laying on 18L:6D). The plasma concentrations of PRL and LH for these same groups are presented in Figure 4. Hens that subsequently exhibited PR had lower plasma levels of T4 at 1 and 2 wk following photostimulation than did hens that remained PS after transfer to 13L:11D. Hens that became PR had significantly lower plasma levels of PRL at 8 and 14 wk after photostimulation than did hens that never became PR. Plasma concentrations of LH, T3, and the T3/T4 ratio did not differ significantly among these groups. Peak egg production for the flock occurred during the fifth week following photostimulation.

DISCUSSION

This study clearly demonstrates that turkey hens may exhibit rPR during the reproductive cycle. When we reduced the photoperiod from 18 to 13 h of light at 19 wk

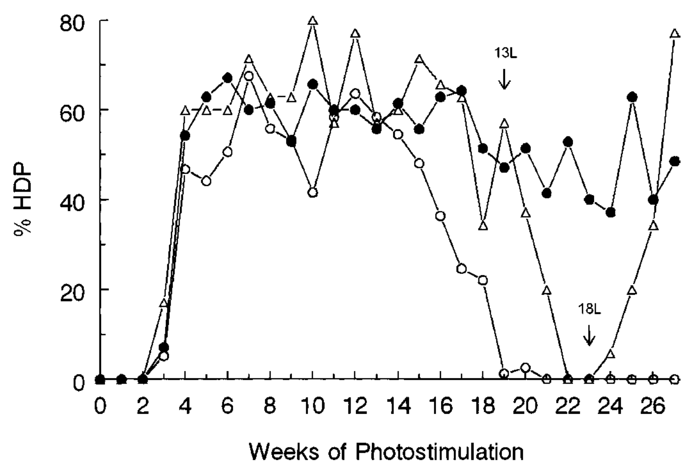

FIGURE 2. Percentage hen-day egg production (%HDP) of turkey hens that: (●) remained photosensitive throughout a reproductive cycle (n = 10); (△) were relatively photorefractory (PR) after 19 wk of photostimulation (n = 5); or (○) became absolutely PR between 12 and 19 wk after photostimulation (n = 11). Photosensitive and relatively PR hens received a photoperiod of 18L:6D until 19 wk (arrow), then 13L:11D until 23 wk when the photoperiod was returned to 18L:6D (arrow). Absolutely PR hens received 18L:6D throughout the experiment.

[3]Diagnostics Products Corp., Los Angeles, CA.

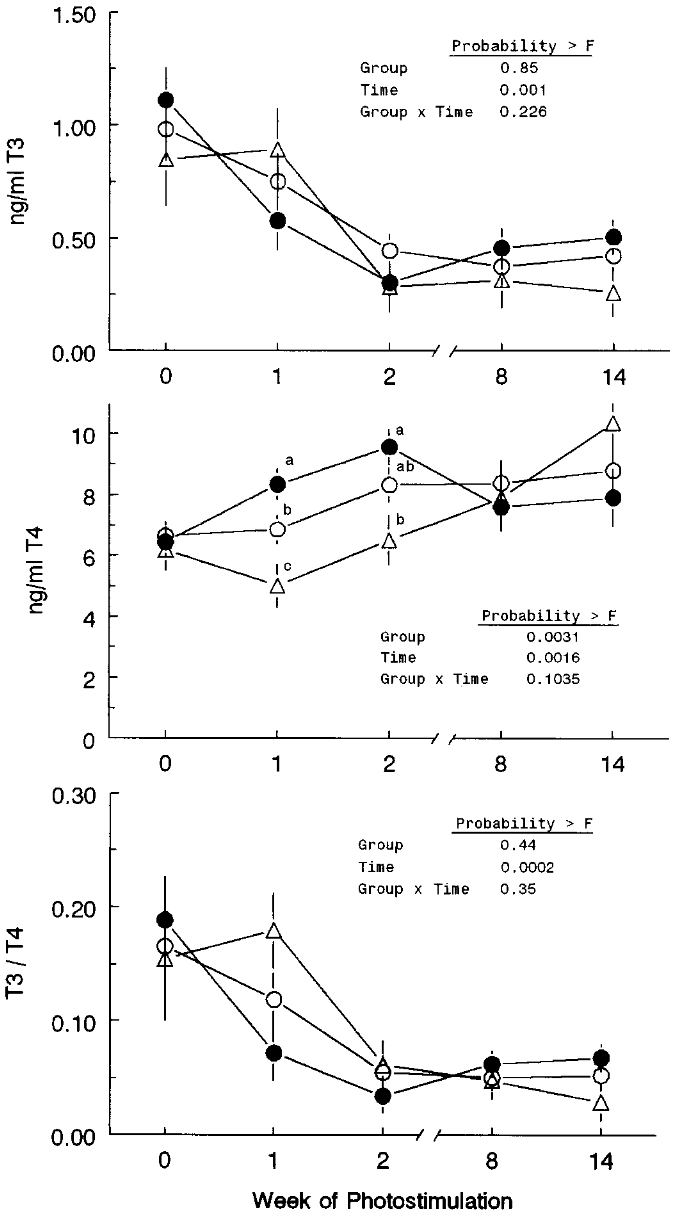

FIGURE 3. Mean (± SEM) plasma concentrations of triiodothyronine (T3), thyroxine (T4), and the ratio of T3/T4 concentrations, of turkey hens that (●) remained photosensitive throughout a reproductive cycle (n = 10); (△) were relatively photorefractory (PR) after 19 wk of photostimulation (n = 5); or (○) became absolutely PR between 12 and 19 wk after photostimulation (n = 11). Means identified by different letters within a week are significantly different ($P \leq 0.05$).

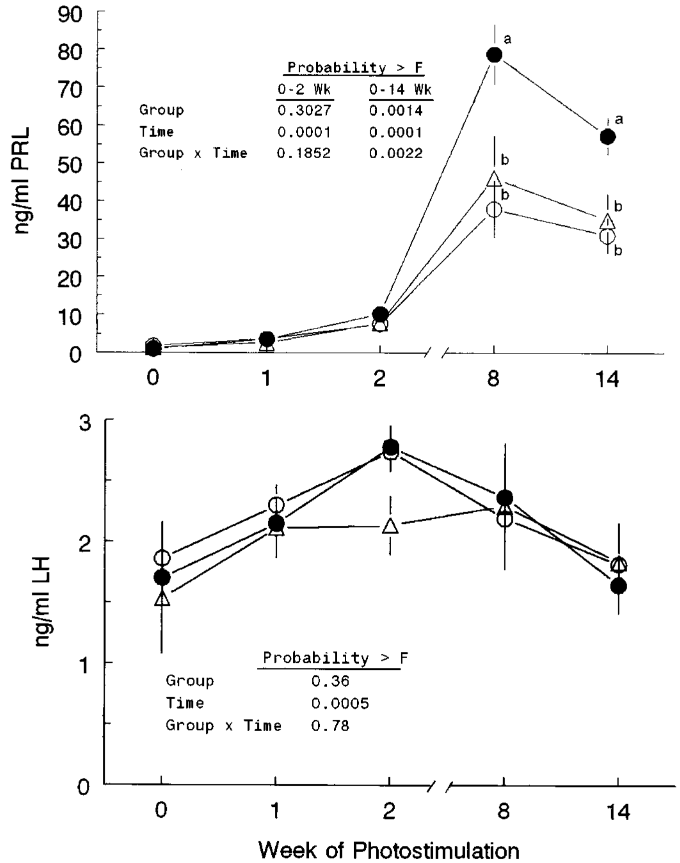

FIGURE 4. Mean (± SEM) plasma concentrations of prolactin (PRL) and luteinizing hormone (LH) of turkey hens that (●) remained photosensitive throughout a reproductive cycle (n = 10); (△) were relatively photorefractory (PR) after 19 wk of photostimulation (n = 5); or (○) became absolutely PR between 12 and 19 wk after photostimulation (n = 11). Means identified by different letters within a week are significantly different ($P \leq 0.05$).

after photostimulation, 27.5% of the hens ceased laying. The ovaries of rPR hens regressed to a similar state as those found in aPR hens. However, re-exposure to a long photoperiod resulted in recrudescence of the ovary and a resumption of egg production in 41.7% of the rPR individuals tested. The individuals who resumed lay were, therefore, similar in their PR responses to Japanese quail, a rPR species that does not show aPR (Nicholls et al., 1988). We also observed a low incidence of aPR in our hens (15.1%). Siopes (2001) reported that the incidence of PR in a different strain of turkey hens was 59 and 89% in two trials and that all individuals who expressed PR did so within 25 wk of photostimulation. Indeed, our PS group showed no evidence of PR at 27 wk after photostimulation, despite a reduction in photoperiod at 19 wk. It seems clear that the incidence of PR in turkey hens can be quite variable and that rPR does occur in turkey hens, as suggested by Siopes (2001). Our observation that 58.3% of rPR hens progressed to aPR is in agreement with the suggestion that rPR is a transitional state between PS and aPR (Follett and Nicholls, 1984; Nicholls et al., 1988; Bentley et al., 1997).

One objective of our experiment was to assess changes in plasma hormone levels early in the reproductive season in relation to the subsequent reproductive status of the hen. To date, most evidence of endocrine programming of PR in birds has been derived from studies of seasonal breeding wild birds. Our earlier observations suggested that turkey hens, unlike other species studied to date, may exhibit seasonal reproductive responses that range from an early onset of aPR to no PR response at all within a single, unmanipulated flock (Siopes, 2001). This range in expression of PR allows a retrospective comparison of the association between early levels of hormones thought to be involved in programming of PR with subsequent PR outcome.

A clear, permissive role of thyroid hormones in PR has been strongly indicated by experiments showing that PR never occurs in thyroidectomized wild birds and that replacement of T4 restores normal seasonal reproduction (for review see Dawson et al., 2001). In these species, T4 appears to permit programming of seasonality within the central nervous system, which occurs within the first few weeks of photostimulation (Bentley, 1997; Wilson and Reinert, 2000). PRL, once suspected of directly inducing PR, is now proposed to have a role in fine-tuning the end of the breeding season by enhancing gonadal regression (Dawson et al., 2001). Our results directly compare early hormone levels among flockmates that remained definitively PS after 27 wk of reproduction with those who became aPR or rPR. To our knowledge, this is the first such direct endocrine comparison of these three states within a single avian species.

In contrast to present theories concerning the relationship of PRL to PR, we found circulating PRL levels following peak egg production to be markedly reduced in hens that subsequently became rPR or aPR compared to hens that remained PS. This finding is consistent with earlier reports by Lein and Siopes (1989) and Proudman (1998). We must emphasize that our experiment excluded birds that exhibited incubation behavior, which results from hyperprolactinaemia (El Halawani and Rozenboim, 1993). Prolactin levels of laying hens typically peak around peak egg production and decline thereafter (Proudman, 1998). Our findings would suggest that hens that will subsequently become PR reach a lower peak in PRL secretion during the early egg production cycle than those that will remain PS. Lein and Siopes (1989) reported a similar response. Arguing against low PRL levels being a causative factor in PR are the observations that immunization of turkeys (El Halawani et al., 1995, 2000) and starlings (Bentley, 1997) against vasoactive intestinal peptide, the PRL-releasing factor, suppresses PRL levels without promoting or preventing PR. It seems likely that the PRL difference observed between PR and PS hens in our study is a correlated response associated with some other physiological difference between PR and PS hens, rather than being a primary causative factor. Further studies are necessary to determine whether low circulating PRL levels following peak egg production may be used as a predictor of which hens will become PR.

Our results also showed that hens destined to become PR later in the reproductive cycle exhibited lower plasma levels of T4 1 and 2 wk after photostimulation than hens that did not show rPR or aPR at the end of the reproductive cycle. Because current theory holds that presence of T4 is required for subsequent PR, we might have anticipated very low T4 levels in our PS group and higher T4 levels in birds that became PR. However, T4 is thought only to allow PR to occur, it does not actively cause PR (Bentley, 1997). It seems likely that the turkey hen, perhaps through genetic selection for egg production, has developed mechanisms that inhibit the factors that drive PR. Indeed, selection for rapid growth and egg production would likely favor mechanisms that would extend reproduction in the presence of an active thyroid gland. Our finding that T4 levels were higher in demonstrably PS hens is consistent with this concept. Our results, therefore, suggest that the role of the thyroid in programming PR is less consistent in the turkey than in wild birds. The turkey may provide a good model for studying the factors that drive the PR process.

We conclude that turkey hens exhibit a range of seasonal reproductive behavior within a flock, including aPR, rPR and extended periods of PS. The presence of long daylengths, thyroid hormones, and PRL did not assure expression of PR. Plasma levels of T4 early in the reproductive cycle were within the expected range but differed between PR and PS hens. Egg production prior to onset of PR is not a predictor of whether a bird will exhibit PR, but PRL levels after peak egg production may be predictive of a tendency to exhibit PR.

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REFERENCES

Bacon, W. L., and J. Y. Long. 1996. Secretion of luteinizing hormone during a forced molt in turkey hens. Poult. Sci. 75:1579–1586.

Bentley, G. E. 1997. Thyroxine and photorefractoriness in starlings. Poult. Avian Biol. Rev. 8:123–139.

Bentley, G. E., A. Dawson, and A. R. Goldsmith. 2000. Lack of gonadotrophin-releasing hormone (GnRH) neuron response to decreasing photoperiod in thyroidectomized male starlings (*Sturnus vulgaris*). J. Exp. Zool. 287:74–79.

Bentley, G. E., A. R. Goldsmith, A. Dawson, L. M. Glennie, R. T. Talbot, and P. J. Sharp. 1997. Photorefractoriness in European starlings (*Sturnus vulgaris*) is not dependent upon the long-day-induced rise in plasma thyroxine. Gen. Comp. Endocrinol. 107:428–438.

Dawson, A., and A. R. Goldsmith. 1983. Plasma prolactin and gonadotrophins during gonadal development and the onset of photorefractoriness in male and female starlings (*Sturnus vulgaris*) on artificial photoperiods. J. Endocrinol. 97:253–260.

Dawson, A., V. M. King, G. E. Bentley, and G. F. Ball. 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythms 16:365–380.

El Halawani, M. E., and I. Rozenboim. 1993. The ontogeny and control of incubation behavior in turkeys. Poult. Sci. 72:906–911.

El Halawani, M. E., J. L. Silsby, I. Rozenboim, and G. R. Pitts. 1995. Increased egg production by active immunization against vasoactive intestinal peptide in the turkey (*Meleagris gallopavo*). Biol. Reprod. 52:179–183.

El Halawani, M. E., S. E. Whiting, J. L. Silsby, G. R. Pitts, and Y. Chaiseha. 2000. Active immunization with vasoactive intestinal peptide in turkey hens. Poult. Sci. 79:349–354.

Follett, B. K., and T. J. Nicholls. 1984. Photorefractoriness in Japanese quail: Possible involvement of the thyroid gland. J. Exp. Zool. 232:573–580.

Lien, R. J., and T. D. Siopes. 1989. Turkey plasma thyroid hormone and prolactin concentrations throughout an egg laying cycle and in relation to photorefractoriness. Poult. Sci. 68:1409–1417.

Nicholls, T. J., A. R. Goldsmith, and A. Dawson. 1988. Photorefractoriness in birds and in comparison with mammals. Physiol. Rev. 68:133–176.

Proudman, J. A. 1998. Circulating prolactin levels at the end of the photophase and the end of the scotophase throughout the reproductive cycle of the turkey hen. Poult. Sci. 77:303–308.

Proudman, J. A., and H. Opel. 1981. Turkey prolactin: Validation of a radioimmunoassay and measurement of changes associated with broodiness. Biol. Reprod. 25:573–580.

SAS Institute Inc. 1990. SAS STAT User's Guide Release 6.08. SAS Institute, Inc., Cary, NC.

Sharp, P. J. 1993. Photoperiodic control of reproduction in the domestic hen. Poult. Sci. 72:897–905.

Sharp, P. J. 1996. Strategies in avian breeding cycles. Anim. Reprod. Sci. 42:505–512.

Siopes, T. 1997. Transient hypothyroidism reinitiates egg laying in turkey breeder hens: Termination of photorefractoriness by propylthiouracil. Poult. Sci. 76:1776–1782.

Siopes, T. D. 2001. Temporal characteristics and incidence of photorefractoriness in turkey hens. Poult. Sci. 80:95–100.

Wilson, F. E. 2001. A test of the hypothesis that T3 is the "seasonality" thyroid hormone in American tree sparrows (*Spizella arborea*): Intracerebroventricular infusion of iopanoic acid, an inhibitor of T3 synthesis and degradation. J. Comp. Physiol. 171:113–119.

Wilson, F. E., and B. D. Reinert. 2000. Thyroid hormone acts centrally to programme photostimulated male American tree sparrows (*Spizella arborea*) for vernal and autumnal components of seasonality. J. Neuroendocrinol. 12:87–95.